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Supplemental information

Allosteric SHP2 inhibition increases apoptotic dependency on BCL2 and synergizes with venetoclax in *FLT3*- and *KIT*-mutant AML

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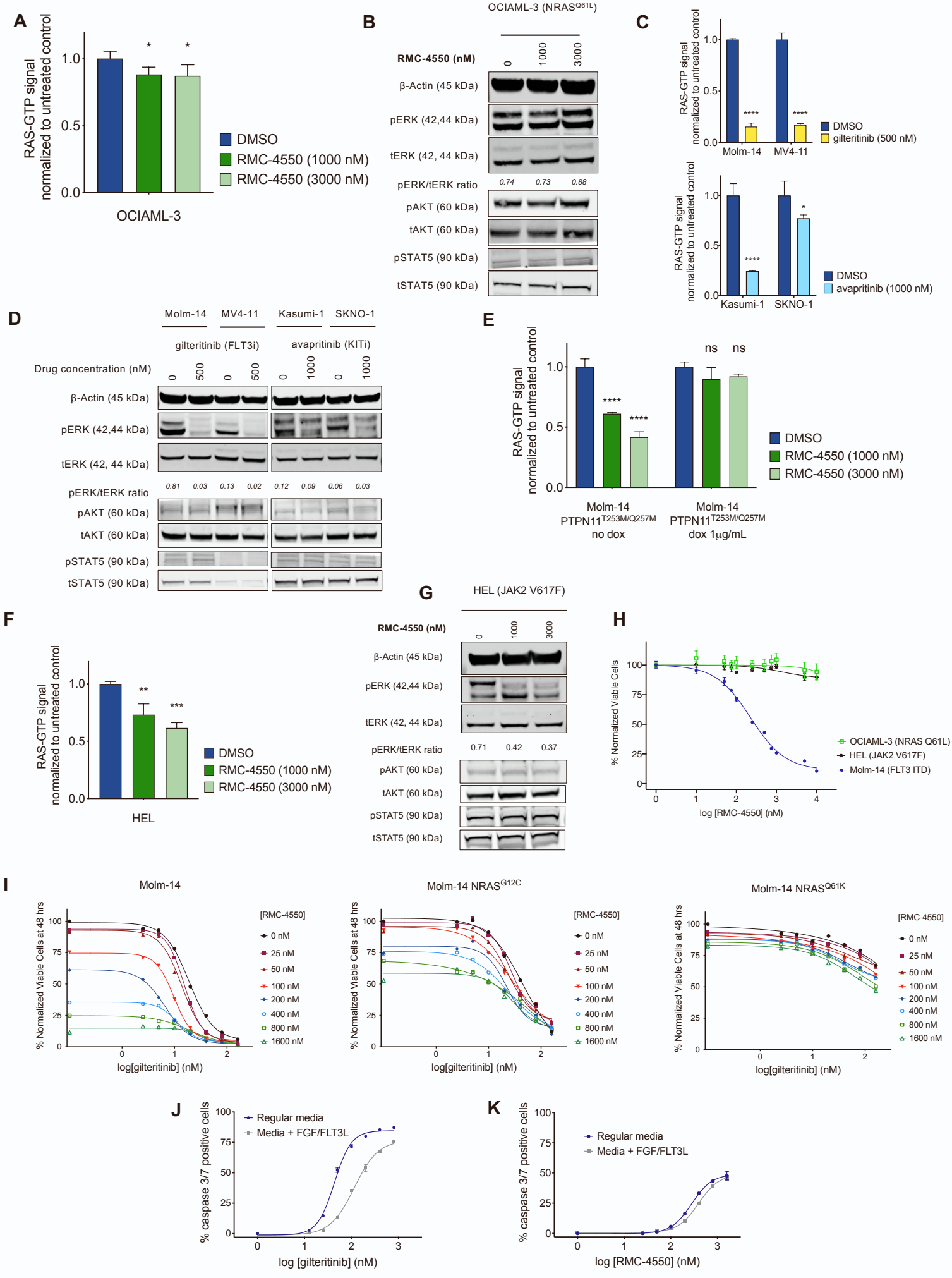
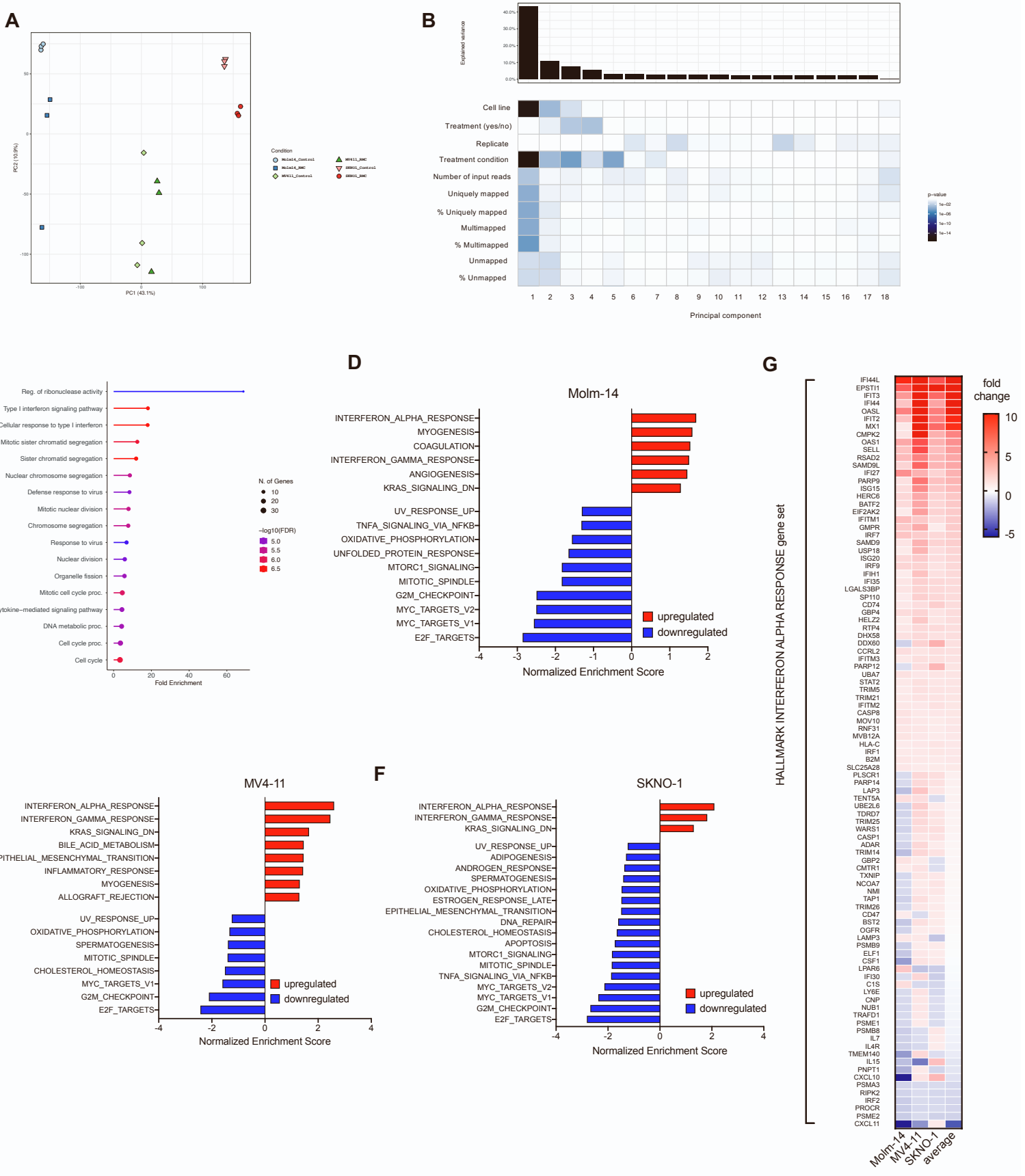
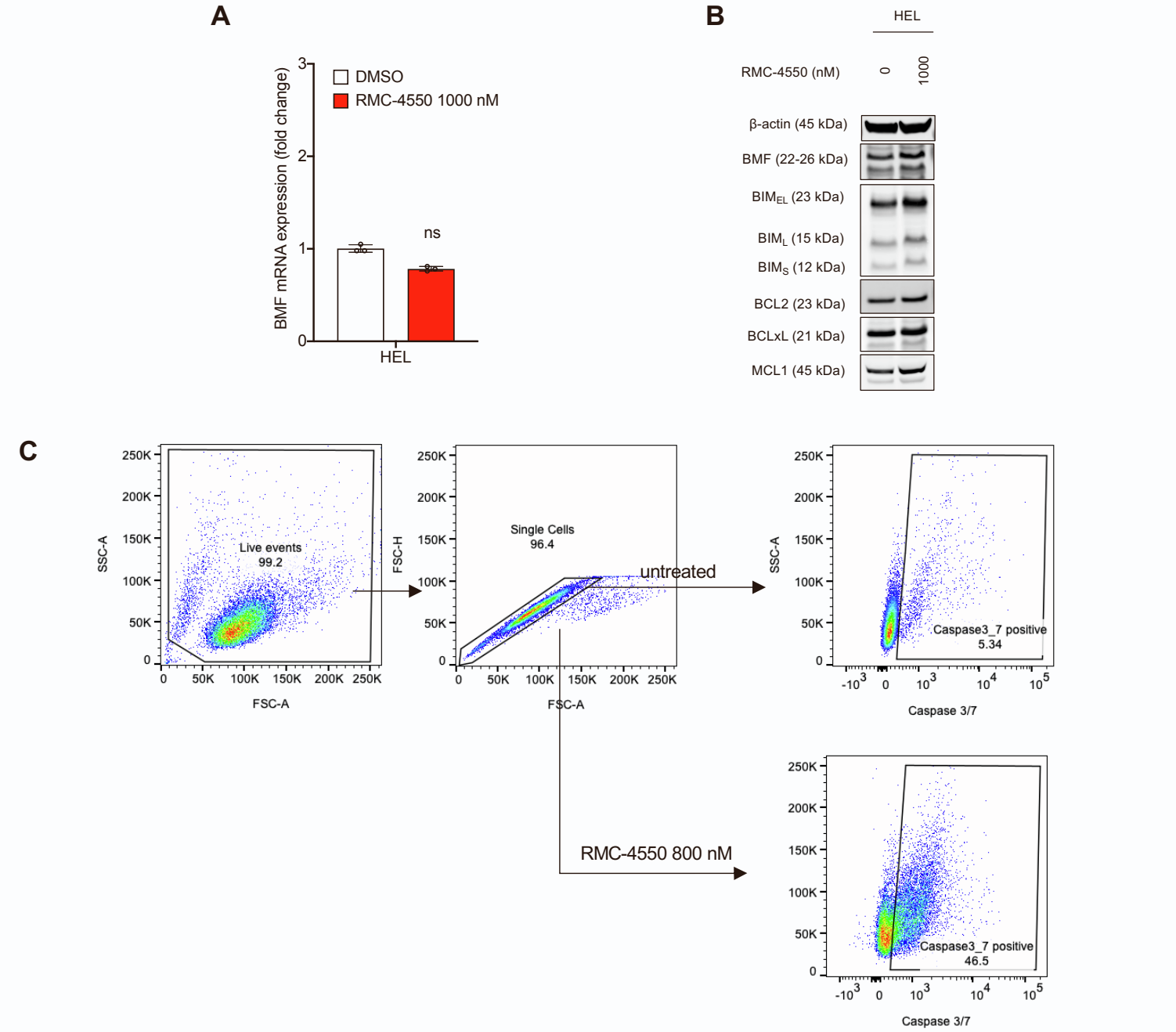

A
RAS-GTP signal normalized to untreated control
1.0
0.5
0.0
*
*
DMSO
RMC-4550 (1000 nM)
RMC-4550 (3000 nM)
OCIAML-3
B
OCIAML-3 (NRASQ61L)
RMC-4550 (nM)
0
1000
3000
β-Actin (45 kDa)
pERK (42,44 kDa)
tERK (42, 44 kDa)
pERK/tERK ratio
0.74
0.73
0.88
pAKT (60 kDa)
tAKT (60 kDa)
pSTAT5 (90 kDa)
tSTAT5 (90 kDa)
C
RAS-GTP signal normalized to untreated control
1.0
0.5
0.0
****
****
Molm-14
MV4-11
DMSO
gilteritinib (500 nM)
RAS-GTP signal normalized to untreated control
1.0
0.5
0.0
****
*
Kasumi-1
SKNO-1
DMSO
avapritinib (1000 nM)
D
Molm-14
MV4-11
Kasumi-1
SKNO-1
gilteritinib (FLT3i)
avapritinib (KITi)
Drug concentration (nM)
0
500
0
500
0
1000
0
1000
β-Actin (45 kDa)
pERK (42,44 kDa)
tERK (42, 44 kDa)
pERK/tERK ratio
0.81
0.03
0.13
0.02
0.12
0.09
0.06
0.03
pAKT (60 kDa)
tAKT (60 kDa)
pSTAT5 (90 kDa)
tSTAT5 (90 kDa)
E
RAS-GTP signal normalized to untreated control
1.0
0.5
0.0
****
****
ns
ns
DMSO
RMC-4550 (1000 nM)
RMC-4550 (3000 nM)
Molm-14 PTPN11T253M/Q257M no dox
Molm-14 PTPN11T253M/Q257M dox 1μg/mL
F
RAS-GTP signal normalized to untreated control
1.0
0.5
0.0
**
***
DMSO
RMC-4550 (1000 nM)
RMC-4550 (3000 nM)
HEL
G
HEL (JAK2 V617F)
RMC-4550 (nM)
0
1000
3000
β-Actin (45 kDa)
pERK (42,44 kDa)
tERK (42, 44 kDa)
pERK/tERK ratio
0.71
0.42
0.37
pAKT (60 kDa)
tAKT (60 kDa)
pSTAT5 (90 kDa)
tSTAT5 (90 kDa)
H
% Normalized Viable Cells
100
75
50
25
0
0
1
2
3
4
log [RMC-4550] (nM)
OCIAML-3 (NRAS Q61L)
HEL (JAK2 V617F)
Molm-14 (FLT3 ITD)
I
Molm-14
Molm-14 NRASG12C
Molm-14 NRASQ61K
% Normalized Viable Cells at 48 hrs
100
75
50
25
0
0
1
2
log[gilteritinib] (nM)
[RMC-4550]
0 nM
25 nM
50 nM
100 nM
200 nM
400 nM
800 nM
1600 nM
J
% caspase 3/7 positive cells
100
75
50
25
0
0
1
2
3
log [gilteritinib] (nM)
Regular media
Media + FGF/FLT3L
K
% caspase 3/7 positive cells
100
75
50
25
0
0
1
2
3
log [RMC-4550] (nM)
Regular media
Media + FGF/FLT3L

Supplementary figure S1. SHP2 inhibition has anti-leukemic activity in RTK-driven AML cell lines. Related to Figure 1. A, Colorimetric detection of RAS-GTP levels in OCIAML-3 cells exposed for 90 minutes to RMC-4550. Data represent means of three technical replicates, error bars represent standard deviation (SD); two-tailed ANOVA with Tukey's correction for multiple comparisons was used for statistical analysis (****$p \leq 0.0001$, ***$p \leq 0.001$, **$p \leq 0.01$, *$p \leq 0.05$, ns, non-significant). **B,** Western blot analysis of OCIAML-3 cell line exposed for 90 minutes to the indicated doses of RMC-4550. Total protein extracts were resolved on a 10% Bis-Tris gel and subjected to immunoblot analysis with the indicated antibodies; actin was used as loading control. For phospho-ERK expression, band intensities from images were normalized to a total ERK control and are shown underneath the relevant bands. **C,** Colorimetric detection of RAS-GTP levels in Molm-14 and MV4-11 cell lines exposed for 90 minutes to RMC-4550 and Kasumi-1 and SKNO-1 cell lines exposed to avapritinib respectively. Data is represented similarly to 1A. **D,** Western blot analysis of Molm-14, MV4-11, Kasumi-1 and SKNO-1 cell lines exposed for 90 minutes to the indicated doses of gilteritinib and avapritinib respectively. **E,** Colorimetric detection of RAS-GTP levels in Molm-14 doxycycline-inducible *PTPN11*$^{T253M/Q257}$ cells exposed for 90 minutes to RMC-4550. 1ug/mL of doxycycline was used to induce the *PTPN11* mutation expression. Data is represented similarly to 1A. **F,** Colorimetric detection of RAS-GTP levels in HEL cells exposed for 90 minutes to RMC-4550. Data is represented similarly to 1A. **G,** Western blot analysis of HEL cell line exposed for 90 minutes to the indicated doses of RMC-4550. **H,** Dose-response curves representing relative proliferation of HEL cells after 48 hours of exposure to serial doses of RMC-4550. **I,** Dose-response curves representing relative proliferation of Molm-14, Molm-14 *NRAS*^{G12C} and Molm-14 *NRAS*^{Q61K} cells after 48 hours of exposure to serial doses of gilteritinib and RMC-4550. **J, K,** Dose-response curves representing apoptosis after 24 hours of treatment with indicated doses of gilteritinib and RMC-4550 in Molm-14 cells cultured in either regular growth medium or medium supplemented with 10 ng/mL FGF2 and 10 ng/mL FLT3-L. Data represented as mean ± SD of three technical replicates.


A
PC1 (43.1%)
PC2 (10.9%)
Condition
Molm14_Control
Molm14_RMC
MV411_Control
MV411_RMC
SKNO1_Control
SKNO1_RMC
B
Explained variance
Cell line
Treatment (yes/no)
Replicate
Treatment condition
Number of input reads
Uniquely mapped
% Uniquely mapped
Multimapped
% Multimapped
Unmapped
% Unmapped
Principal component
p-value
C
Reg. of ribonuclease activity
Type I interferon signaling pathway
Cellular response to type I interferon
Mitotic sister chromatid segregation
Sister chromatid segregation
Nuclear chromosome segregation
Defense response to virus
Mitotic nuclear division
Chromosome segregation
Response to virus
Nuclear division
Organelle fission
Mitotic cell cycle proc.
Cytokine−mediated signaling pathway
DNA metabolic proc.
Cell cycle proc.
Cell cycle
Fold Enrichment
N. of Genes
−log10(FDR)
D
Molm-14
INTERFERON_ALPHA_RESPONSE
MYOGENESIS
COAGULATION
INTERFERON_GAMMA_RESPONSE
ANGIOGENESIS
KRAS_SIGNALING_DN
UV_RESPONSE_UP
TNFA_SIGNALING_VIA_NFKB
OXIDATIVE_PHOSPHORYLATION
UNFOLDED_PROTEIN_RESPONSE
MTORC1_SIGNALING
MITOTIC_SPINDLE
G2M_CHECKPOINT
MYC_TARGETS_V2
MYC_TARGETS_V1
E2F_TARGETS
upregulated
downregulated
Normalized Enrichment Score
E
MV4-11
INTERFERON_ALPHA_RESPONSE
INTERFERON_GAMMA_RESPONSE
KRAS_SIGNALING_DN
BILE_ACID_METABOLISM
EPITHELIAL_MESENCHYMAL_TRANSITION
INFLAMMATORY_RESPONSE
MYOGENESIS
ALLOGRAFT_REJECTION
UV_RESPONSE_UP
OXIDATIVE_PHOSPHORYLATION
SPERMATOGENESIS
MITOTIC_SPINDLE
CHOLESTEROL_HOMEOSTASIS
MYC_TARGETS_V1
G2M_CHECKPOINT
E2F_TARGETS
upregulated
downregulated
Normalized Enrichment Score
F
SKNO-1
INTERFERON_ALPHA_RESPONSE
INTERFERON_GAMMA_RESPONSE
KRAS_SIGNALING_DN
UV_RESPONSE_UP
ADIPOGENESIS
ANDROGEN_RESPONSE
SPERMATOGENESIS
OXIDATIVE_PHOSPHORYLATION
ESTROGEN_RESPONSE_LATE
EPITHELIAL_MESENCHYMAL_TRANSITION
DNA_REPAIR
CHOLESTEROL_HOMEOSTASIS
APOPTOSIS
MTORC1_SIGNALING
MITOTIC_SPINDLE
TNFA_SIGNALING_VIA_NFKB
MYC_TARGETS_V2
MYC_TARGETS_V1
G2M_CHECKPOINT
E2F_TARGETS
upregulated
downregulated
Normalized Enrichment Score
G
HALLMARK INTERFERON ALPHA RESPONSE gene set
fold change
Molm-14
MV4-11
SKNO-1
average

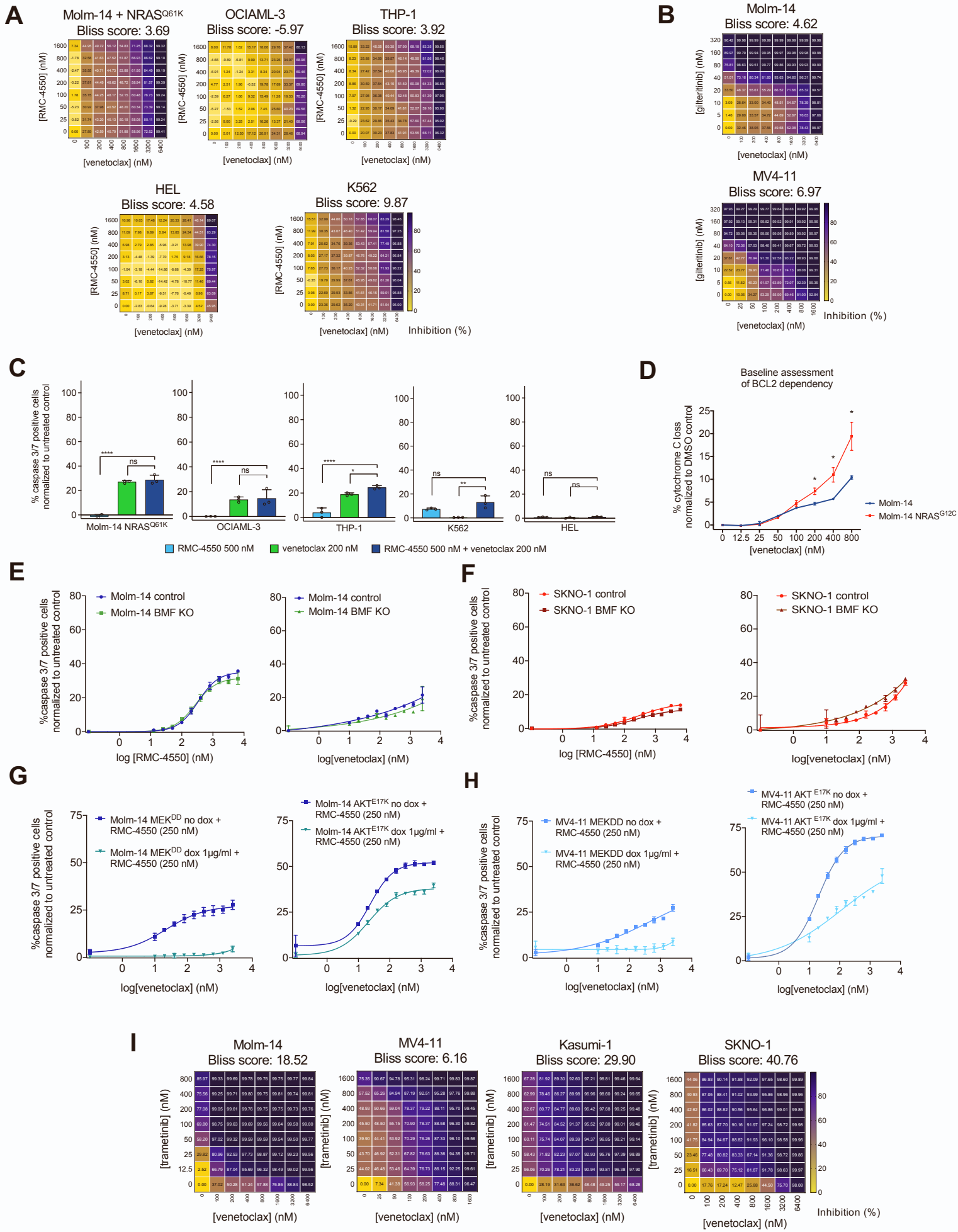
Supplementary figure S2. SHP2 inhibition alters the transcriptomic profile of RTK-driven AML. Related to Figure 2. A, Principal component analysis (PCA) scatter plot for the first two principal components from the normalized reads data. Each symbol corresponds to individually treated cell cultures of each cell line (n=3), treated with either DMSO or RMC-4550. **B,** Plots showing the significance of association tests between the available covariates and the principal components from the data. The statistical test used are: ANOVA for a categorical covariate, and a Spearman correlation test for a continuous covariate. p-values are shown as continuous measures (gradients of blue) using a statistical significance threshold of $p < 0.05$ and a Benjamini-Hochberg adjustment for multiple testing. **C,** Gene ontology (GO) Biological Process analysis of the 132 differentially expressed genes depicted in main figure 2B. The analysis was performed using the ShinyGO v0.77 app at https://bioinformatics.sdstate.edu/go and illustrates top 17 enrichments, selected by FDR cutoff of 0.05 and sorted by Fold Enrichment. **D-F,** Barplots representing enriched hallmark gene sets as resulted from GSEA analysis performed in each individual cell line. Data represents gene sets enriched with a nominal p value > 0.05 and sorted by Normalized Enrichment Scores. **G,** heatmap representing relative expression of genes in the Hallmark Interferon Alpha Response (MSigDB) gene set in all three cell lines.



	Molm-14	MV4-11	Kasumi-1	SKNO-1
Max. caspase3/7 %	45.62	34.4	16.75	1.96
SD	4.15	10.15	0.69	0.8

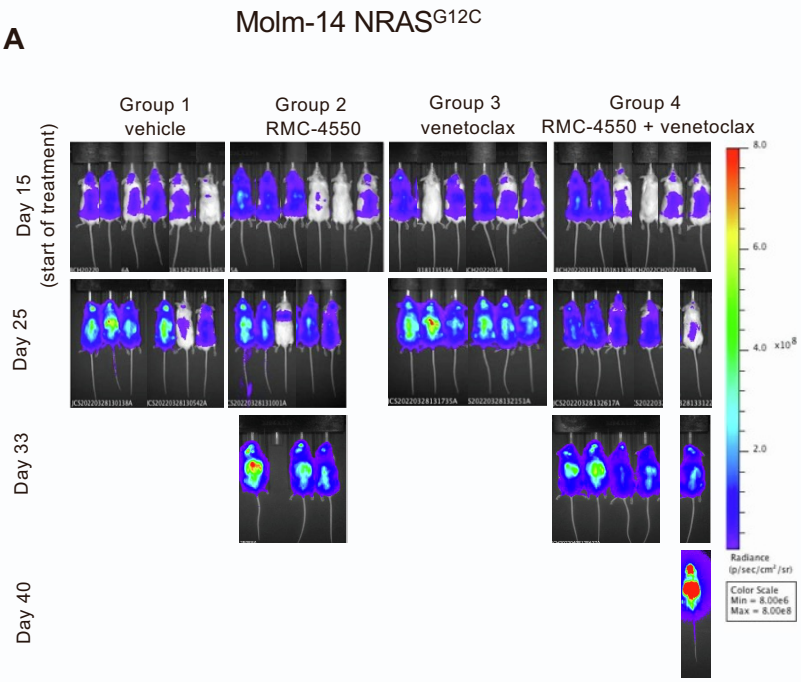
	Molm-14	MV4-11	Kasumi-1	SKNO-1
Max. caspase3/7 %	33.83	20.71	7	1.26
SD	3.09	7.39	0.21	0.2

Supplementary figure S3. Related to Figure 3. Apoptosis induction in *FLT3*- and *KIT*- mutant AML cell lines. Related to Figure 3. A, RT-qPCR data representing gene expression fold change of BMF gene in HEL cell line after 24 hours of treatment with RMC-4550. Data represent mean ± SD of three technical replicates; Wilcoxon test was used for statistical significance. **B,** Western blot analysis of pro- and anti-apoptotic proteins in HEL cells exposed to 1000 nM of RMC-4550 for 24 hours; actin was used as loading control. **C,** Representative gating strategy used to measure caspase3/7 positive cells. **D-E**, Dose-response curves showing apoptosis measured by flow cytometry in Molm-14, MV4-11, Kasumi-1 and SKNO-1 cell lines after 24 hours of exposure to increasing doses of RMC-4550 and trametinib respectively. Caspase3/7 positive cells were gated and normalized to untreated controls. Data represent means ± SD of three technical replicates per drug condition.

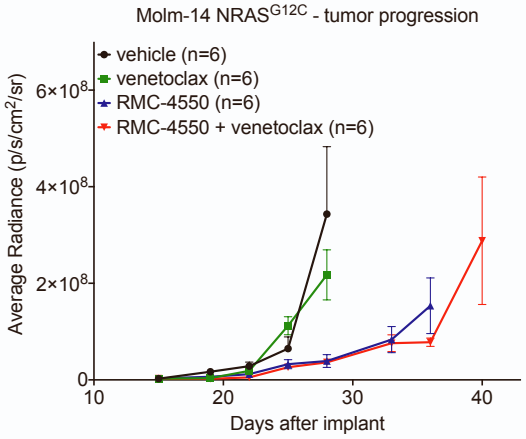

A
Molm-14 + NRASQ61K
Bliss score: 3.69
OCIAML-3
Bliss score: -5.97
THP-1
Bliss score: 3.92
HEL
Bliss score: 4.58
K562
Bliss score: 9.87
[RMC-4550] (nM)
[venetoclax] (nM)
Inhibition (%)
B
Molm-14
Bliss score: 4.62
MV4-11
Bliss score: 6.97
[gilteritinib] (nM)
[venetoclax] (nM)
Inhibition (%)
C
% caspase 3/7 positive cells normalized to untreated control
Molm-14 NRASQ61K
OCIAML-3
THP-1
K562
HEL
ns
RMC-4550 500 nM
venetoclax 200 nM
RMC-4550 500 nM + venetoclax 200 nM
D
Baseline assessment of BCL2 dependency
% cytochrome C loss normalized to DMSO control
Molm-14
Molm-14 NRASG12C
[venetoclax] (nM)
E
%caspase 3/7 positive cells normalized to untreated control
Molm-14 control
Molm-14 BMF KO
log [RMC-4550] (nM)
log[venetoclax] (nM)
F
SKNO-1 control
SKNO-1 BMF KO
log [RMC-4550] (nM)
log[venetoclax] (nM)
G
Molm-14 MEKDD no dox + RMC-4550 (250 nM)
Molm-14 MEKDD dox 1µg/ml + RMC-4550 (250 nM)
Molm-14 AKTE17K no dox + RMC-4550 (250 nM)
Molm-14 AKTE17K dox 1µg/ml + RMC-4550 (250 nM)
log[venetoclax] (nM)
H
MV4-11 MEKDD no dox + RMC-4550 (250 nM)
MV4-11 MEKDD 1µg/ml + RMC-4550 (250 nM)
MV4-11 AKT E17K no dox + RMC-4550 (250 nM)
MV4-11 AKT E17K dox 1µg/ml + RMC-4550 (250 nM)
log[venetoclax] (nM)
I
Molm-14
Bliss score: 18.52
MV4-11
Bliss score: 6.16
Kasumi-1
Bliss score: 29.90
SKNO-1
Bliss score: 40.76
[trametinib] (nM)
[venetoclax] (nM)
Inhibition (%)

Supplementary figure S4. Combination therapy with RMC-4550 and venetoclax has synergistic activity in RTK-driven AML cell line models. Related to Figure 4. A-B, Dose-response matrices representing normalized cell viability inhibition following 48 hours of treatment with increasing doses of RMC-4550 and venetoclax (A) and gilteritinib and venetoclax (B) in the indicated cell lines. Synergy scores were computed using Bliss method within Synergy Finder v3.0 software. **C,** Apoptosis measured by flow cytometry after 24 hours for treatment with RMC-4550 and venetoclax at indicated concentrations. Results depict caspase3/7 positive cells normalized to untreated controls. Data represent mean ± SD of three technical replicates; statistical analysis was performed using one-way ANOVA test with Dunnett correction for multiple comparisons. **D,** iBH3 profiling data representing normalized cytochrome C release following exposure to increasing doses of BCL2 inhibitor venetoclax in Molm-14 and Molm-14 $NRAS^{G12C}$ cells. Data represents mean ± SD of three technical replicates per drug condition and *t* test with Sidak-Bonferrroni correction for multiple comparisons was used for statistical significance (****$p \leq 0.0001$, ***$p \leq 0.001$, **$p \leq 0.01$, *$p \leq 0.05$, ns, non-significant). **E-F,** Dose-response curves of apoptosis measured after 24 hours of treatment with either RMC-4550 or venetoclax in Molm-14 and SKNO-1 BMF KO cells compared to control cells. Data represent mean ± SD of three technical replicates per drug condition, normalized to untreated controls; **G-H,** Dose-response curves of apoptosis measured after 24 hours of treatment with RMC-4550 and venetoclax in Molm-14 and MV4-11 cell lines expressing MEK^{DD} and AKT^{E17K} doxycycline-inducible mutations, compared to uninduced control cells. Data represent mean ± SD of three technical replicates per drug condition, normalized to untreated controls; **I,** Dose-response matrices representing normalized cell viability inhibition following 48 hours of treatment with increasing doses of trametinib and venetoclax in the indicated cell lines. Synergy scores were computed using Bliss method within Synergy Finder v3.0 software.

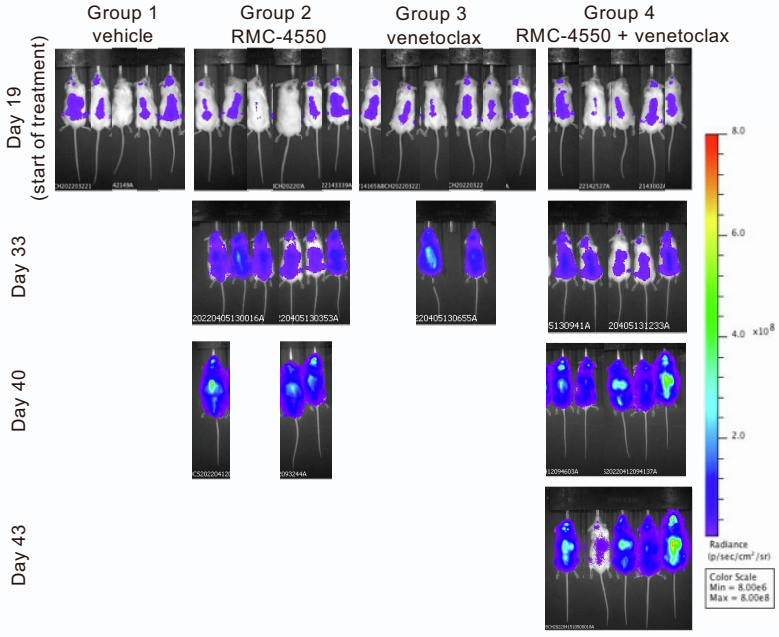
A


Molm-14 NRAS^{G12C}
Group 1
vehicle
Group 2
RMC-4550
Group 3
venetoclax
Group 4
RMC-4550 + venetoclax
Day 15
(start of treatment)
Day 25
Day 33
Day 40
Radiance
(p/sec/cm²/sr)
Color Scale
Min = 8.00e6
Max = 8.00e8

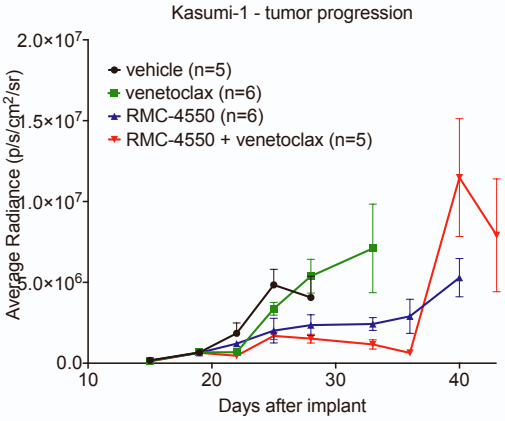
C


Molm-14 NRAS^{G12C} - tumor progression
vehicle (n=6)
venetoclax (n=6)
RMC-4550 (n=6)
RMC-4550 + venetoclax (n=6)
Average Radiance (p/s/cm²/sr)
Days after implant

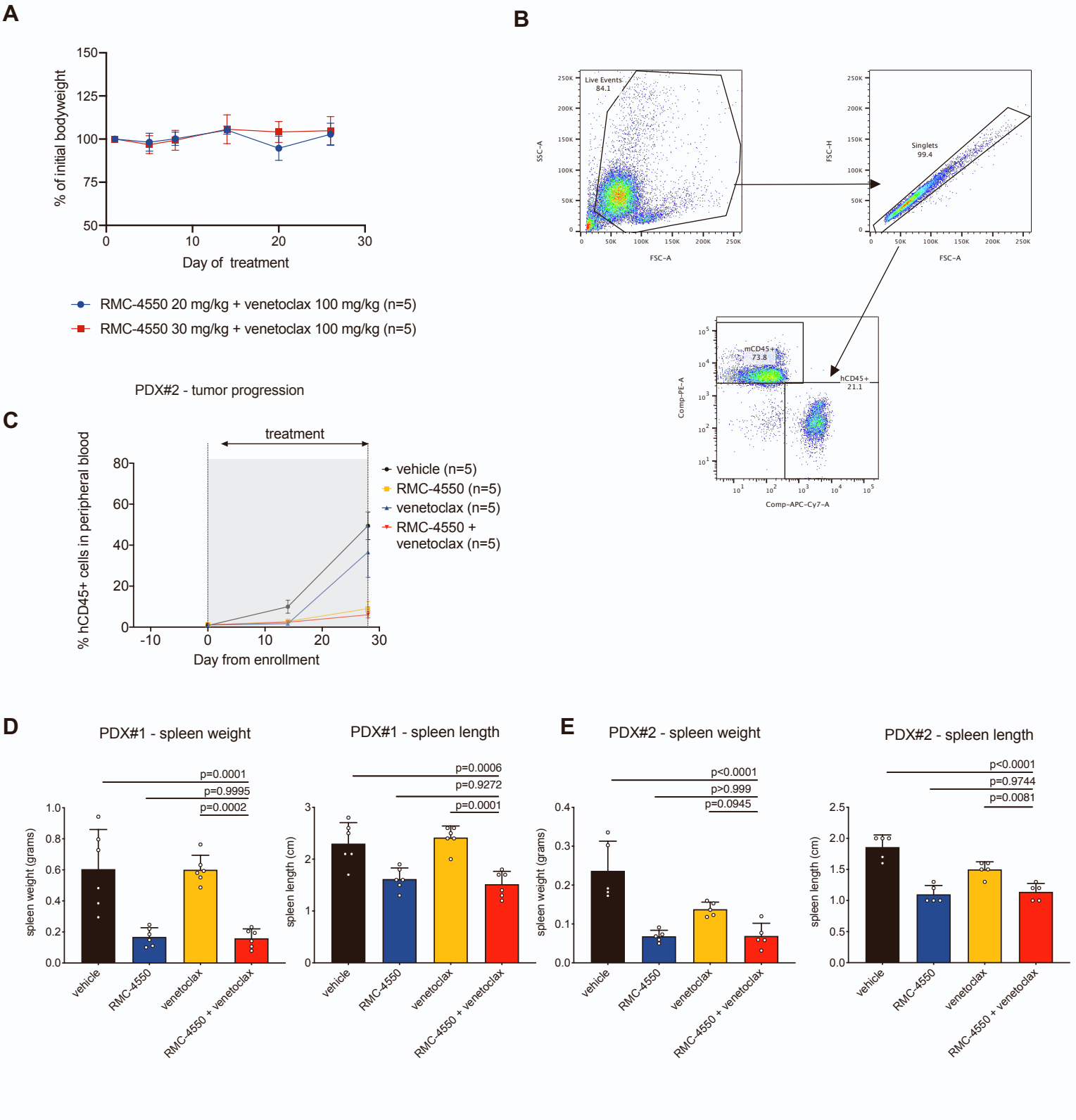
B


Kasumi-1
Group 1
vehicle
Group 2
RMC-4550
Group 3
venetoclax
Group 4
RMC-4550 + venetoclax
Day 19
(start of treatment)
Day 33
Day 40
Day 43
Radiance
(p/sec/cm²/sr)
Color Scale
Min = 8.00e6
Max = 8.00e8

D


Kasumi-1 - tumor progression
vehicle (n=5)
venetoclax (n=6)
RMC-4550 (n=6)
RMC-4550 + venetoclax (n=5)
Average Radiance (p/s/cm²/sr)
Days after implant

Supplementary figure S5. Combination therapy with RMC-4550 and venetoclax is effective *in vivo* in CDX AML models. Related to Figure 5. A, B, Representative images of *in vivo* BLI assessment of NSG mice engrafted with luciferase-tagged Molm-14 *NRAS*^{G12C} (n=6/group, **A**) and Kasumi-1 (n=5/group, **B**) cells over the course of the trial. **C, D,** Quantification of BLI data from the Molm-14 *NRAS*^{G12C} (**C**) and Kasumi-1 (**D**) CDX studies, data represents mean ± SD (n=5/group).


A
% of initial bodyweight
Day of treatment
RMC-4550 20 mg/kg + venetoclax 100 mg/kg (n=5)
RMC-4550 30 mg/kg + venetoclax 100 mg/kg (n=5)
B
Live Events
84.1
SSC-A
FSC-A
Singlets
99.4
FSC-H
mCD45+
73.8
hCD45+
21.1
Comp-PE-A
Comp-APC-Cy7-A
C
PDX#2 - tumor progression
treatment
% hCD45+ cells in peripheral blood
Day from enrollment
vehicle (n=5)
RMC-4550 (n=5)
venetoclax (n=5)
RMC-4550 +
venetoclax (n=5)
D
PDX#1 - spleen weight
p=0.0001
p=0.9995
p=0.0002
spleen weight (grams)
PDX#1 - spleen length
p=0.0006
p=0.9272
p=0.0001
spleen length (cm)
E
PDX#2 - spleen weight
p<0.0001
p>0.999
p=0.0945
spleen weight (grams)
PDX#2 - spleen length
p<0.0001
p=0.9744
p=0.0081
spleen length (cm)
vehicle
RMC-4550
venetoclax
RMC-4550 + venetoclax

Supplementary figure S6. Simultaneous SHP2 and BCL2 inhibition is effective in FLT3-mutant PDX AML models. Related to Figure 6. A, Body weight changes over the course of 28 days of treatment with RMC-4550 at 20 mg/kg compared to 30 mg/kg and venetoclax 100 mg/kg. Data represents mean ± SD (n=5/group). **B,** Representative gating strategy for flow cytometry assessment of hCD45+ cells **C,** quantification of hCD45+ cells over the course of treatment in the four treatment groups in the PDX #2 study. **D, E,** Measurements of spleen weight and length at the study termination in PDX #1 (n=6/group, **D**) and PDX #2 – n=5/group, **E**). Data represents mean ± SD; one-way ANOVA with Tukey correction for multiple comparisons was used for statistical analysis (****$p \leq 0.0001$, ***$p \leq 0.001$, **$p \leq 0.01$, *$p \leq 0.05$, ns, non-significant).

Cell line	*Signaling mutation*	*IC_{50} (nM)*
Molm-14	FLT3-ITD	146.3
MV4-11	FLT3-ITD	120
Kasumi-1	KIT N822K	192.9
SKNO-1	KIT N822K	479.7
U937	PTPN11 G60R	>10000
OCIAML-3	NRAS Q61L	>10000
THP-1	NRAS G12D	>10000
NOMO-1	NRAS G13D	>10000
K562	BCR-ABL1	2086
HEL	JAK2 V617F	>10000

Supplementary Table S1 – IC_{50} values of RMC-4550 in parental AML cell lines. Related to Figure 1.

Figure 1E	*Cell line*	*Treatment condition*	*Normalized pERK/tERK ratio*	
	Molm-14	untreated	1.00	
		RMC-4550 1000 nM	0.50	
		RMC-4550 3000 nM	0.29	
	MV4-11	untreated	1.00	
		RMC-4550 1000 nM	0.35	
		RMC-4550 3000 nM	0.28	
	Kasumi-1	untreated	1.00	
		RMC-4550 1000 nM	0.19	
		RMC-4550 3000 nM	0.19	
	SKNO-1	untreated	1.00	
		RMC-4550 1000 nM	0.14	
		RMC-4550 3000 nM	0.11	
Figure S1B, S1G	*Cell line*	*Treatment condition*	*Normalized pERK/tERK ratio*	
	OCIAML-3	untreated	1.00	
		RMC-4550 1000 nM	0.99	
		RMC-4550 3000 nM	1.18	
	HEL	untreated	1.00	
		RMC-4550 1000 nM	0.60	
		RMC-4550 3000 nM	0.53	
Figure S1D	*Cell line*	*Treatment condition*	*Normalized pERK/tERK ratio*	
	Molm-14	untreated	1.00	
		gilteritinib 500 nM	0.04	
	MV4-11	untreated	1.00	
		gilteritinib 500 nM	0.18	
	Kasumi-1	untreated	1.00	
		avapritinib 1000 nM	0.72	
	SKNO-1	untreated	1.00	
		avapritinib 1000 nM	0.61	
Figure 3C	*Cell line*	*Treatment condition*	*Normalized BMF/actin*	*Normalized MCL1/actin*
	Molm-14	untreated	1.00	1.00
		RMC-4550 1000 nM	6.32	0.62
	MV4-11	untreated	1.00	1.00

		RMC-4550 1000 nM	1.79	0.76
	Kasumi-1	untreated	1.00	1.00
		RMC-4550 1000 nM	1.47	1.03
	SKNO-1	untreated	1.00	1.00
		RMC-4550 1000 nM	2.80	0.57
	OCIAML-3	untreated	1.00	1.00
		RMC-4550 1000 nM	0.95	0.82
Figure S3B	HEL	untreated	1.00	1.00
		RMC-4550 1000 nM	1.23	1.46
Figure 4C	*Cell line*	*Treatment condition*	*Normalized BMF/actin*	
	Molm-14	untreated	1.00	
		RMC-4550 1000 nM	6.84	
	Molm-14 BMF KO	untreated	1.00	
		RMC-4550 1000 nM	0.63	
	SKNO-1	untreated	1.00	
		RMC-4550 1000 nM	4.50	
	SKNO-1 BMF KO	untreated	1.00	
		RMC-4550 1000 nM	0.38	
Figure 4F	*Cell line*	*Treatment condition*	*Normalized MCL1/actin*	*Normalized pERK/tERK ratio*
	Molm-14	untreated	1.00	1.00
		90 min RMC-4550 1000 nM	1.16	0.20
		90 min venetoclax 200 nM	1.32	0.85
		90 min combination	1.25	0.14
		24 hrs RMC-4550 1000 nM	0.47	2.37
		24 hrs venetoclax 200 nM	1.05	0.26
		24 hrs combination	0.12	0.26
	Molm-14 NRAS G12C	untreated	1.00	1.00
		90 min RMC-4550 1000 nM	0.97	0.73
		90 min venetoclax 200 nM	1.19	0.92
		90 min combination	1.02	0.87
		24 hrs RMC-4550 1000 nM	0.38	0.86
		24 hrs venetoclax 200 nM	0.60	0.63
		24 hrs combination	0.25	0.56
	SKNO-1	untreated	1.00	1.00
		90 min RMC-4550 1000 nM	1.09	0.07
		90 min venetoclax 200 nM	1.16	1.02
		90 min combination	1.49	0.06
		24 hrs RMC-4550 1000 nM	0.83	0.31
		24 hrs venetoclax 200 nM	1.75	0.72
		24 hrs combination	0.72	0.28

Supplementary Table S2 – Normalized quantified signal from western blots. Related to Figures 1, 3, 4, S1, S3.

Sample/Local ID	*Age*	*Gender*	*Disease status*	*Previous treatment*	*Clinical genotype*
PDX #1 / HM0007	24	F	Relapsed AML	7+3, HiDAC x2, alloHSCT	FLT3-ITD
PDX #2 / CD33CART_005	33	M	Relapsed AML	7+3+midostaurin, HiDAC+midostaurin, CLAG-M, alloHSCT, Ara-C, gilteritinib, sorafenib, azacitidine	FLT3-ITD NUP-98-NSD1 fusion WT1 indels (in trans)
CFU-AML #1	69	M	Relapsed AML	7+3, HiDAC	FLT3-ITD, NPM1-mut
CFU-AML #2	60	M	AML arising from atypical CML-BC	Hydroxyurea, Ara-C	FLT3-ITD
CFU-AML #3	32	F	AML at diagnosis	N/A	FLT3-ITD
CFU-AML #4	69	F	AML post MDS	Hydroxyurea	FLT3-ITD, NPM1-mut, DNMT3A-mut, IDH2-mut
CFU-HD #1	58	M	Healthy donor	N/A	N/A
CFU-HD #2	30	M	Healthy donor	N/A	N/A
CFU-HD #3	29	M	Healthy donor	N/A	N/A
CFU-HD #4	28	M	Healthy donor	N/A	N/A

Supplementary Table S3 – Clinical data of primary samples used in PDX and CFU experiments. Related to Figure 6.